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IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT: Michel IBEA et al

SERIAL NO.: 08/702,~~[illegible]~~ 113 GROUP:

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FOR: CHIMERIC FATTY BODY-GRF ANALOGS WITH INCREASED BIOLOGICAL POTENCY

DECLARATION UNDER 37 C.F.R. 1.132

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I Dr. Paul Brazeau of 4054 Avenue du Parc Lafontaine, Montréal, Québec, Canada do hereby declare the following.

I am co-inventor of the above-captioned application. As such, I am fully knowledgable of the present invention and the disclosure of the specification.

As evidenced by my curriculum vitae, a copy of which is enclosed herewith, I am considered as one-skilled in the art of the present invention. I am also fully knowledgeable of the references of Gaudreau et al., Coy et al., Felix et al., Bercu et al., Kann et al., Recker and Clark, as cited by the Examiner.

The comparative experiments presented below, which were conducted under my direct supervision and control, demonstrate the unexpected and unobovious advantages over the compounds of Gaudreau et al. and Coy et al. There is further no suggestion of these advantages in the remaining cited references.

Introduction

The present invention is drawn to a chimeric fatty body-pro-GRF analog with increased biological potency, of the following general formula:

A1-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-Tyr-Arg-Lys-Val-Leu-A15-Gln-Leu-A18-Ala-Arg-Lys-Leu-Leu-A24-Asp-Ile-A27-A28-Arg-A30-R_0

wherein,

A1 is Tyr or His;

A2 is Val or Ala;

A8 is Asn or Ser;

A15 is Ala or Gly;

A18 is Ser or Thr;

A24 is Gln or His;

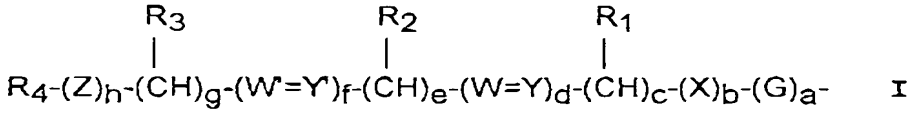
A27 is Met, Ile or Nle;

A28 is Ser or Asp;

A30 is any amino acid sequence of 1 to 15 residues;

R_0 is NH2;

wherein A1 is N- or O-anchored by a hydrophobic tail of the following general formula I:



I

wherein,

G is a carbonyl, a phosphonyl, a sulfuryl or a sulfinyl group;

X is an oxygen atom, sulfur atom or an amino group (NH);

(W=Y) represents cis or trans ($CH=CR_5$);

(W'=Y') represents cis or trans (CH=CR6);

Z is an oxygen or a sulfur atom;

R_1, R_2 and R_3, independently, are selected from a hydroxyl group, a hydrogen atom, and a linear or branched C_1-C_6 alkyl group;

R_4 is a hydroxyl group, a hydrogen atom or a linear or branched C_5-C_9 alkyl group;

R_5 and R_6, independently, are a hydrogen atom or a linear or branched C_1-C_4 alkyl group;

a is 0 or 1;

b is 0 or 1;

c is 0 to 8;

d is 0 or 1;

e is 0 to 8;

f is 0 or 1;

g is 0 to 8;

h is 0 to 1;

wherein the sume of d + f ≥ 1 and the sum of a, b, c, d, e, f, g and h is such that the hydrophobic tail of formula I has a linear main chain of between 5 and 7 atoms (C, O and/or S).

Thus, the present invention is drawn to alkene based analogs of chimeric fatty body-pro-GRF compounds having a linear main chain of between 5-7 carbons.

Experimental procedures

Experiment 1 - Superior activity of alkene based main chains

Methods:

Thirty-two (32) pigs were randomly distributed into 4 experimental groups (n = 8 pigs per group). Each group received two daily S.C. administrations of the following treatments (volume: 3 ml, S.C. injection).

group 1: saline
group 2: (Hexanoyl)$_0$ hGRF(1-29)NH_2
group 3: (Hexenoyl *trans*-2)$_0$ hGRF(1-29)NH_2
group 4: (Hexenoyl *trans*-3)$_0$ hGRF(1-29)NH_2

Treatments were administered from day 1 to 5. Immediately before the injections, one blood sample was collected from each animal, and additional blood samples were collected on days 6, 7 and 8 in group 2 only.

Blood samples were allowed to clot, serum was harvested by centrifugation and sent to Notre-Dame Hospital for IGF-I assays.

Results:

As seen in Table I and Figures 1 and 2, hexenoyl compounds (both *trans*-2 and *trans*-3) remain active much longer than the corresponding hexanoyl compound, as evidenced by the serum concentrations of IGF-1.

Experiment 2 - Effects of linear chain main chain length

Thirty two (32) pigs were cannulated (a catheter surgically implanted in one jugular vein) within one week before the study. Cannulated animals were randomly distributed into 4 experimental groups (n = 8 pigs per group).

group 1: hGRF(1-29)NH_2
group 2: $(Octanoyl)_0$ hGRF(1-29)NH_2
group 3: $(Hexanoyl)_0$ hGRF(1-29)NH_2
group 4: $(Butyryl)_0$ hGRF(1-29)NH_2

All groups were subjected to the following sequence of events:

Day 1: single IV administration (4 μg/kg)
Blood samples for pGH assay were collected every 20 min from 1 hour before to 5 hours after test articles injections, with additional samples 10 and 30 min after injection (n = 21 samples per profile).
From day 8 to day 12, twice daily SC administration of test articles (20 μg/kg) to all animals:
day 8: SC injection at 9:00 am and 6:00 pm
day 9: SC injection at 9:00 am and 6:00 pm
day 10: SC injection at 9:00 am and 6:00 pm
day 11: SC injection at 9:00 am and 6:00 pm

day 12: SC injection at 9:00 am and 6:00 pm

Blood samples for pGH assay were collected on day 8 (first injection of test articles) from 1 hour before to 6 hours after test article injections (every 20 min; n = 22 samples per profile).

Additional blood samples were collected on days 9, 10, 11, 12, 13 (before test article administration) for IGF-I measurement.

Blood samples were allowed to clot overnight at 4°C. Serum was harvested by centrifugation, stored at -20°C and sent to Notre-Dame Hospital for pGH and IGF-I assays.

Results:

As seen in Table 2 and Figure 3, chimeric fatty body-pro-GRF compounds having a linear main chain of 6 carbons have unexpected superior activity over compounds with either a shorter (butyryl = 4 carbons) or a longer (octanoyl = 8 carbons) main chain length, as evidenced by the serum concentration of IGF-1.

Conclusions

Neither Gaudreau et al. nor Coy et al. disclose fatty body-pro-GRF compounds having a double bond and a linear main chain of between 5-7 carbons. Gaudreau et al. disclose alkane based compounds of 3, 4, 6 or 8 carbons. However, in Gaudreau et al. there is no suggestion of alkene based compounds or any advantages of compounds having 5 to 7 carbons. In addition, Coy et al. only

disclose acetyl based compounds.

The experiments described herein clearly demonstrate that alkene fatty body pro-GRF compounds having 5 to 7 carbons in the main chain have unexpected, unobvious activity over those of disclosed by Gaudreau et al. and Coy et al.

As such, in my opinion the superior activity and advantages of the compounds of the present invention are in no way disclosed or suggested by the cited prior art references and the present invention is therefore unobvious over the prior art.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This 15th day of September, 1996

Dr. Paul Brazeau

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